

Claims

- 1 A nucleic acid molecule encoding a pesticidal fusion
polypeptide comprising (i) a toxin domain; and (ii) a
5 heterologous binding domain capable of binding non-
specifically to a cell membrane without disrupting that
membrane.
- 2 A nucleic acid as claimed in claim 1 wherein the toxin
10 domain is derived from a *Bacillus thuringiensis* cry toxin.
- 3 A nucleic acid as claimed in claim 2 wherein the
Bacillus thuringiensis cry toxin is CryIA(b) or (c).
- 15 ~~4 A nucleic acid as claimed in any one of the preceding
claims wherein the binding domain binds carbohydrate.~~
- 5 A nucleic acid as claimed in claim 4 wherein the binding
domain has galactose or galactosyl affinity.
- 20 ~~6 A nucleic acid as claimed in claim 4 or claim 5 wherein
the binding domain is derived from a lectin.~~
- 7 A nucleic acid as claimed in claim 6 wherein the lectin
25 is a type two ribosome inactivating protein.
- 8 A nucleic acid as claimed in claim 7 wherein the binding
domain is derived from the ricin toxin B chain.
- 30 ~~9 A nucleic acid as claimed in any one of claims 2 to 8
which comprises all or part of Seq ID No 1 (CryIA(b)) or Seq
ID No 2 (CryIA(c)) or a sequence degeneratively equivalent
thereto.~~
- 35 10 A nucleic acid as claimed in any one of claims 2 to 9
which comprises all or part of Seq ID No 3 (RTB1), Seq ID No
4 (RTB2) or Seq ID No 5 (RTB3) or a sequence degeneratively

equivalent thereto.

5 11 A nucleic acid as claimed in claims 9 or claim 10 which comprises the CryIA-RTB combination shown in any one of Seq ID No 6 (CryIA(b)-RTB1); Seq ID No 7 (CryIA(b)-RTB2); Seq ID No 8 (CryIA(b)-RTB3); Seq ID No 9 (CryIA(c)-RTB1); Seq ID No 10 (CryIA(c)-RTB2); or Seq ID No 11 (CryIA(c)-RTB3) or a sequence degeneratively equivalent thereto.

10 12 A nucleic acid as claimed in any one of claims 2 to 8 which comprises a nucleotide sequence which is a homologous variant of any of Seq ID Nos 1 to 11.

15 13 A method of producing the nucleic acid of any one of claims 1 to 12, which method comprises the step of combining a nucleic acid encoding a toxin with a nucleic acid encoding a heterologous binding domain, wherein said binding domain is capable of binding non-specifically to a cell membrane without disrupting it.

20 14 A method as claimed in claim 13 wherein the method further comprises the step of modifying the sequence of the toxin or binding domain by way of addition, insertion, deletion or substitution of one or more nucleotides in the
25 nucleic acid.

15 A method as claimed in claim 14 wherein the modification of the sequence causes an alteration in the codon usage of the sequence.

30

30 16 A recombinant vector comprising a nucleic acid as claimed in any one of claims 1 to 12.

35 17 A vector as claimed in claim 16 wherein the nucleic acid of any one of claims 1 to 12 is operably linked to a promoter.

18 A vector as claimed in claim 17 which is an inducible

promoter which is switched on in response to an elicitor or other plant signal which is triggered in response to predation.

- 5 19 A vector as claimed in any one of claims 16 to 18 which is a baculovirus vector or a vector suitable for use in a plant.
- 10 20 A method for transforming a host cell which method includes the step of introducing a vector of any one of claims 16 to 19 into the cell and causing or allowing recombination between the vector and the cell genome to introduce the nucleic acid into the genome.
- 15 21 A host cell containing the nucleic acid of any one of claims 1 to 12 or the vector of any one of claims 16 to 19.
- 20 22 A host cell transformed with the nucleic acid of any one of claims 1 to 12 or the vector of any one of claims 16 to 19.
- 23 A host cell as claimed in claim 21 or claim 22 which is a plant cell.
- 25 24 A host cell as claimed in claim 23 wherein the plant is a monocot plant.
- 30 25 A host cell as claimed in claim 24 wherein the monocot is maize or rice.

- 35 26 A process for producing a transgenic plant, which process comprises the steps of:
- (a) performing the method of claim 20 to produce a transformed plant cell;
- (b) regenerating a plant from said transformed host cell.

sub 7.. 27 A plant obtainable by the process of claim 26, which
A16 comprises the host cell of any one of claims 23 to 25.
cont

5 28 A plant which is a clone, selfed or hybrid progeny, or
other descendant of the plant of claim 27, and which
comprises the host cell of any one of claims 23 to 25.

10 29 A plant as claimed in claim 27 or claim 28 which is a
monocot.

30 30 A plant as claimed in claim 29 wherein the monocot is
maize or rice.

sub 7 15 31 A part or propagule of the plant of any one of claims 27
A7 to 30 which comprises the host cell of any one of claims 23
to 25.

20 32 A method of influencing or affecting the toxicity of a
plant to a pest, which method includes the step of causing or
allowing expression from a nucleic acid of any one of claims
1 to 12 in the plant.

25 33 A pesticidal fusion polypeptide encoded by the nucleic
acid of any one of claims 1 to 12.

30 34 A method for producing the polypeptide of claim 33 which
method comprises the step of causing expression from a
nucleic acid of any one of claims 1 to 12 in a suitable host
cell.

35 35 A composition comprising the polypeptide of claim 33
plus at least one additional component.

35 36 A commodity which has been treated with the composition
of claim 35 such that it has a reduced susceptibility to
attack by a pest.

sub 7 37 A method for controlling pests comprising the use of the
AB polypeptide of claim 33.

38 A method of assessing the toxicity of a polypeptide to a
5 pest species comprising:

(i) introducing a nucleic acid encoding said polypeptide
into a host cell from that species,

(ii) causing or allowing the nucleic acid to be
expressed in a host cell from that species,

10 (iii) observing the viability of the cell and
correlating the results of the observation with the toxicity
of the polypeptide, wherein the viability is determined by
assessing esterase activity or membrane integrity.

sub 15 7 39 A method as claimed in claim 37 or claim 38 wherein the
AR pest is a species of insect.

40 A method as claimed in claim 39 wherein the species is
selected from *Lepidoptera*, *Coleoptera*, *Culicidae*, *Simuliidae*,

20 *Hymenoptera*, *Homoptera*, *Orthoptera* and *Diptera*.

sub 7 41 An oligonucleotide selected from the group consisting
B3 of:

LF1=5' CAACAACAAAGGAATTCATGCTGATG 3',

25 LB1=5' GGACACACACACTGCAAGCTTGTAATC 3',

LB2=5' CGGATCCGAAAGCTTCACATCTAACAC 3', or

LB3=5' GCTTGCAAGCTTAGACCATATAGCCC 3'

AT10?